30. The method of claim 13, wherein the immunoglobulin heavy chain comprises at least a portion of a constant region and said constant region is from an IgG heavy chain.

REMARKS

The present invention stems from Applicants pioneering discovery that fully assembled antigen-specific immunoglobulin can be produced in a plant cell. The inventors also were the first to achieve a level of expression that makes possible passive immunization with plant produced antibodies. Plant produced antibodies are useful for systemic protection through administration i.v. as well as localized protection through local administration to a mucosal surface (e.g., lungs, digestive tract, nasopharyngeal cavity, the urogenital system).

The claims are generally directed to methods of passive immunization using antigen-specific immunoglobulin produced in transgenic plants. After amending the claims as set forth above, claims 13, 15-27, 29-82 will be pending in this application. The amendments and the new claims find ample basis in the application as filed. For example, support for "antigen-specific immunoglobulin" is found, for example, at page 10, line 27-33 (emphasis added):

> Immunoglobulin product: A polypeptide, protein or multimeric protein **containing at least the immunologically active portion of an immunoglobulin heavy chain and is thus capable of specifically combining with an antigen**. Exemplary immunoglobulin products are an immunoglobulin heavy chain, immunoglobulin molecules, substantially intact immunoglobulin molecules, any portion of an immunoglobulin that contains the paratope, including those portions known in the art as Fab fragments, Fab' fragment, $F(ab')_2$ fragment and Fv fragment.

Support for immunoglobulin fragments, a heavy chain fragment, or a light chain fragment finds basis throughout the specification. Indeed reference in the above quote to "at least the immunologically active portion" . . . any portion of an immunoglobulin . . . including those portions known in the art" strongly supports that a variety of fragments of

the heavy or the light chain were contemplated, not just those known by proteolytic cleavage. This view is additionally supported by page 3, lines 1-6 (emphasis added) of the specification.

> One of the most useful aspects of using a recombinant expression system for antibody production is the ease with which the antibody can be tailored by molecular engineering. **This allows the production of antibody fragments and single-chain molecules, as well as the manipulation of full-length antibodies**. For example, a side [sic] range of functional recombinant-antibody fragments, such as Fab, F_v, single-chain and single-domain antibodies, may be generated.

This passage indicates that recombinant expression makes possible the production of a variety of antibody fragments including those known from proteolytic processing (e.g., Fab) and those known only by recombinant expression of heavy and light chain fragments (e.g., single chain antibodies).

Thus, in view of the above, the amendments raise no issue of new matter.

REJECTION UNDER 35 U.S.C. § 102 OVER STOLLE

The rejection of claims 13 and 15-30 under 35 U.S.C. § 102(b) as being allegedly anticipated by Stolle et al., (U.S. 4,748,018) is respectfully traversed. Claim 28 has been cancelled, herein, thus rendering the rejection moot as to this claim.

Relevant Law

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. In re Spada F.2d, 15 USPQ2d 1655 (Fed. Clr, 1990), In re Bond, F.2d , 15 USPQ 1566 (Fed. Clr. 1990). Furthermore, it is incumbent on Examiner to identify wherein each and every facet of the claimed invention is disclosed. Lindemann Maschinen-fabrik Gmbh v. American Hoist and Derrick Co., 730 F.2d,1452, 221 USPQ 481 (Fed. Cir. 1984).

Argument

A feature common to all the rejected claims is the requirement that the immunoglobulin which is used for passive immunization be obtained from transgenic plant cells that contain nucleic acid encoding the immunoglobulin. The claims also require the plant cells to contain immunoglobulin product encoded by the nucleic acid. A further requirement is for the nucleic acid to encode a leader sequence and that the leader sequence form a secretion signal which is cleaved from the immunoglobulin polypeptide (heavy or light chain or H/L single polypeptide) following proteolytic processing, the result of this process being production of an antigen-specific immunoglobulin in a plant cell.

Stolle et al., does not disclose any of these requirements. The reference does not even mention, let alone teach how to prepare antigen-specific immunoglobulin in plant cells. Stolle et al. also does not describe the requirement for the transgenic nucleic acid to encode a leader sequence and for the leader sequence form a secretion signal which is cleaved from the immunoglobulin polypeptide (heavy or light chain) following proteolytic processing. Indeed, the only immunoglobulins described in Stolle et al. are obtained from conventionally immunized birds (see col.7, lines 40-51). Because Stolle et al. fails to disclose or otherwise teach or suggest any of these claim requirements, the reference fails to anticipate the claims as a matter of law.

Accordingly, the examiner is respectfully urged to withdraw the rejection of the claims as allegedly anticipated by Stolle et al.

REJECTION UNDER 35 U.S.C. § 103 OVER DÜRING IN VIEW OF STOLLE

The rejection of claims 13 and 15-30 under 35 U.S.C. § 103(a) as being allegedly unpatentable over a doctoral dissertation by Klaus During ("the During dissertation"), in view of Stolle et al, is respectively traversed. Claim 28 has been cancelled herein, thus rendering the rejection moot as to this claim. All reference herein to the During dissertation will be to the English language translation prepared by Ralph McElroy Translation Company, 910 West Avenue, Austin Texas (Job No. 1596-81522).

Relevant Law

A claimed invention is obvious if the differences between it and the prior art "are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art." 35 U.S.C. § 103 (1994); see also Graham v. John Deere, 383 U.S. 1, 13 (1966). Federal Circuit case law provides that "[t]he consistent criterion for determination of obviousness is whether the prior art would have suggested to one of ordinary skill in the art that this process should be carried out and would have a reasonable likelihood of success, viewed in the light of the prior art." In re Dow Chem., 837 F.2d 469, 473, 5 USPQ2d 1529, 1531 (Fed.Cir.1988). Under the law, there must be a showing of a suggestion, teaching, or motivation to combine the prior art references is an "essential evidentiary component of an obviousness holding." C.R. Bard, Inc. v. M3 Sys. Inc., 157 F.3d 1340, 1352, 48 USPQ2d 1225, 1232 (Fed.Cir.1998). Also required is that the combined teachings have a reasonable expectation of success, viewed in light of the prior art. *See* In re Dow Chemical Co., 837 F.2d 469, 473, 5 USPQ2d 1529, 1531 (Fed.Cir.1988)("Both the suggestion and the expectation of success must be founded in the prior art, not in applicant's disclosure.").

The examiner bears the burden of establishing a *prima facie* case of obviousness. In re Rijckaert, 9 F.3d 1531, 1532, 28 USPQ2d 1955, 1956 (Fed.Cir.1993); In re Oetiker, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed.Cir.1992). This showing must be clear and particular, and broad conclusory statements about the teaching of multiple references, standing alone, are not "evidence." *See* Dembiczak, 175 F.3d at 1000, 50 USPQ2d at 1617. However, the suggestion to combine need not be express and "may come from the prior art, as filtered through the knowledge of one skilled in the art." Motorola, Inc. v. Interdigital Technology Corp., 121 F.3d 1461, 1472, 43 USPQ2d 1481, 1489 (Fed.Cir.1997). Only when the examiner's burden is met does the burden of coming forward with rebuttal argument or evidence shift to applicant. Rijckaert, 9 F.3d at 1532, 28 USPQ2d at 1956.

Argument

To reiterate, the claims all require that immunoglobulin used for passive immunization be obtained from transgenic plant cells that contains nucleic acid encoding the immunoglobulin. Also required is for the plant to contain antigen-specific immunoglobulin product encoded by the nucleic acid. Further required is for the nucleic acid to encode a leader sequence and that the leader sequence forms a secretion signal that is cleaved from the immunoglobulin polypeptide (heavy or light chain or H/L single polypeptide) following proteolytic processing. Finally, the immunoglobulin is expressed in plant cells, it is isolated from the cells and then administered in an appropriate amount to passively immunize an individual.

As will be addressed below, it is respectfully submitted that even in its most favorable light, the During dissertation fails to teach or suggest these requirements and that such deficiency is not cured by Stolle or any other reference of record. In particular, During fails to demonstrate production of immunoglobulin that exhibits binding specificity for a preselected antigen, an essential requirement for passive immunity. Supporting Applicant's position is a declaration under 37 C.F.R. §1.132 by Richard Lerner, M.D., President of the Scripps Research Institute ("the Lerner declaration"), a copy of which is attached herewith.

A. The claims are not obvious because During used a different strategy from Applicants for light chain and heavy chain expression.

The strategy used by During for light and heavy chain expression is different from that which underlies the claimed invention, in particular, the requirement for nucleic acid to encode a heavy and light chain along with a leader sequence for each chain, and the requirement for production of antigen immunoglobulin product encoded by the nucleic acid, and the requirement that the leader sequence forms a secretion signal that is cleaved from the immunoglobulin polypeptide (heavy or light chain or H/L single polypeptide) following proteolytic processing. According to the During dissertation, the nucleic acid encoding the barley alpha amylase signal sequence was inserted directly 5' to the end of the DNA encoding the amino terminal end of the mature heavy chain. In the case of the

light chain, however, During included nucleic acid encoding three additional amino acids (Gly-Ser-Met) between the DNA encoding the leader sequence and the DNA encoding the mature amino terminus of the light chain. Lerner declaration, ¶9. The additional amino acids that would be encoded at the 3' end of the light chain leader sequence constructed by During were unusual, according to Lerner, and it was not clear what effect additional amino acids would have on final processing of the leader. Lerner declaration, ¶¶10 and 11. It is now clear from the art that sequence modifications introduced in the vicinity of a cleavage site have the potential to adversely influence signal processing. Lerner declaration, ¶11. In the opinion of Lerner, During's strategy of adding additional amino acid residues to the cleavage site for the light chain leader likely obscured substrate recognition causing cleavage site ambiguity. Lerner declaration, ¶¶11 and 12.

Thus, it is respectfully submitted that these facts alone evidence that the During dissertation does not teach the claim requirement for proteolytic processing of a leader sequence and assembly of the heavy and light chains to form an antigen-specific immunoglobulin in the plant cell. This deficiency in the teachings of During is not cured by any teaching in Stolle, which deals only with classical methods of animal immunization. It is respectfully submitted, therefore, that no substantive foundation exists upon which to find the claims obvious over the combination of references. Accordingly, the rejection fails on this basis alone.

B. The claims are not obvious because During's assertion of successful antibody expression in plants would not have been believed by the ordinary skilled artisan or, in the alternative, the During dissertation is non-enabling.

1. There was a prejudice in the art against the possibility that plant cells could be used to produce an antigen-specific immunoglobulin

Any analysis of the teachings of the prior art in the context of an obviousness rejection must be made from the perspective of the ordinary skilled artisan. The Lerner declaration goes to great length to properly ascertain this perspective at the time period beginning from the alleged publication date of the During dissertation (July 1988) and up to the earliest priority date of the above captioned patent application (October 27, 1989). The analysis shows that there was a prejudice in the art at the relevant time period against the possibility of using plant cells to process and assemble an antigen-specific immunoglobulin. According to Lerner, it was appreciated by the early 1980s that the biology of antibody expression was complex and varied with the maturation state of the B cell. For example, rearrangement of immunoglobulin chain variable region encoding gene segments is required to form a functional immunoglobulin gene, and rearrangement of the heavy chain occurs before rearrangement of the light chain. In fact, there is an early stage B cell known as the "pre-B cell," characterized in having a productively rearranged heavy chain V gene but not a rearranged light chain V gene. Lerner declaration, ¶3. In contrast, a later stage of B cells is known ("young B cell), characterized in having both the heavy and the light chain V genes productively rearranged and in expressing a full-sized immunoglobulin on the cell surface. *Id.*

Lerner goes on to explain that antibody expression in B cells was understood to be further complicated by the involvement of the BiP protein, known to be involved in heavy chain processing. Lerner declaration, ¶3. A phenomenon called heavy chain toxicity also was appreciated at the time but its mechanism was unknown. Lerner declaration, ¶4. According to Lerner, by the mid 1980s, a prejudice had taken hold in the art against the notion that antigen-specific immunoglobulins could be produced in cells other than mammalian B cells. *Id.*

Although Lerner notes the existence of reports describing expression of an assembled antibody in two microorganisms (i.e., *Saccharomyces cerevisiae* and *E. coli*) he provides substantial reasoning for why the prevailing prejudice in the art would still have existed with respect to producing antigen-specific immunoglobulin in plant cells. Lerner declaration, ¶7. For example, Lerner notes that plant cells were known to be different from mammalian cells and from microorganisms such as *Saccharomyces cerevisiae* and *E.*

coli not only in having a cell wall but also in features related to protein secretion. In addition, Lerner notes that it was not known at the time whether plant cells contained a BiP protein or a functionally equivalent analogue. Lerner concludes from his review of the field that:

> [T]here was a sound basis for a real prejudice in the art against using plants to produce a processed and assembled immunoglobulin which is antigen specific around the time of the During dissertation (*circa* 1988/1989). Were this not the case, then Applicant's invention clearly would not have been roundly hailed in both the scientific literature and in the general press as a significant scientific discovery and medical breakthrough.

Lerner declaration, ¶8 (footnotes removed). It stands to reason, therefore, that the ordinary skilled artisan in the 1988/1989 time frame would have applied this prejudice to any claim purporting to demonstrate processing and assembly of an antigen-specific immunoglobulin in plant cells and would not have accepted such claim unless the proof was well founded. It is respectfully submitted that the teachings of the During declaration with respect to the instant obviousness rejection, must be viewed in light of this prejudice.

2. <u>During's experimental results allegedly supporting immunoglobulin expression are internally inconsistent and are lacking in critical controls</u>

During initially made a light chain only expression vector and evaluated whether plant cells transfected with this vector could express light chains. During, however, failed to detect light chain production in the transfected cells (During dissertation, p. 80, line 2). According to Lerner, this fact would have been disturbing to the ordinary skilled artisan because light chain alone is readily expressed in B cells, and even if During's cells were making a small amount of light chain, albeit at a level below his detectability limit, this would complicate efforts to achieve and detect heavy-light chain assembly. Lerner further points out that an increased relative heavy chain expression, which under the circumstances might be necessary to obtain assembly in view of the low levels of

expressed light chain, conceivably could result in toxicity if plant cells were susceptible to heavy chain toxicity, as was the case for mammalian B cells. These issues would have raised serious questions about During's chances for success and would have required additional proof for any alleged success to be accepted in the art.

Although During appreciated that his expression system was suboptimal, he proceeded to attempt expression of both a heavy and light chain from a single expression vector. Anticipating a threshold detectability problem, During utilized a pre-enrichment step prior to Western blotting (i.e., indirect Western) of transgenic plant extracts. Lerner declaration, ¶14. Lerner points out that During's need for an indirect Western also would have been disturbing to the ordinary skilled artisan because direct Western blotting was known to be a very sensitive technique that had previously been successfully used to demonstrate foreign host expression (including plant expression of antibodies as disclosed in the instant patent application). *Id.*

The Examiner is referred to the Lerner declaration § 15 for details of During's indirect Western results. It is significant that During claims to observe light chain production with the dual chain vector (but not with the light chain only vector used earlier) but was unable to detect heavy chains by either direct or indirect Western blotting. *Id.* During's assertion that he has detected the presence of assembled B1-8 antibody in the plant cells is based, according to Lerner, on faulty circular logic.

> To conclude as he does from the Western results that assembled B1-8 antibody was present in the plant extract, During must infer that which he is attempting to prove, that fully assembled antibody must have been present in the extract for light chain to have been enriched following binding to the NP hapten immunoabsorbent. As will be seen below, this faulty circular reasoning is open to alternative explanations that directly conflict with During's conclusion.

Lerner declaration, ¶ 15. Lerner goes on to discuss numerous other reasonable explanations for the results that During did not address, let alone attempt to exclude. Notably, During fails to exclude the real possibility that light chain may have been enriched by the NP immunoabsorbent even if the light chain were not assembled with a heavy chain. During's failure to detect heavy chains by direct and indirect Western blotting is

consistent with this possibility. As summarized by Lerner, there was much that During could have done (but failed to do) to exclude alternative artifactual explanations for his Western blotting data.

> For example, During could have directly demonstrated that heavy chain was absolutely required for light chain binding during the pre-enrichment step. Alternatively, or in addition, During could have used biosynthetic radiolabeling of plant cells in combination with Western blotting to prove that a heavy chain was in fact co-enriched with light chain. This method is well known in the art and was previously used to demonstrate foreign protein expression. Biosynthetic radiolabeling also helps to control for stripping of antibody during a low pH elution of an antibody immunoabsorbent column (i.e., the Ls136 adsorbent), a problem encountered with CNBr. Since During employed low pH elution and CNBr linkage, he should have provided controls to address this potential problem.

Lerner declaration, ¶ 16 (footnotes removed).

The During dissertation also evaluated antibody expression in his plants using a second technique referred to as "tissue printing." In this technique, a leaf is pressed against a membrane in order to bind proteins in the leaf to the membrane, and the membrane is probed by immunological reagents as in Western blotting. The During dissertation describes that light chain, heavy chain and "aggregated B1-8" antibody were detected by tissue printing. Although During asserts that these results support his conclusion of successful immunoglobulin assembly, Lerner is of the opinion that the tissue printing experiment are easily susceptible to alternative explanations because they lack controls which are essential to conclude that binding of an immunological reagent is antigen-specific. Lerner declaration, ¶ 17. Lerner bases his opinion not only on his own experience as a scientist and immunologist for more than 30 years but also on the scientific literature. With respect to the latter, Lerner points out that the types of controls lacking in the During dissertation were used by others who previous to During demonstrated expression in yeast of the same B1-8 antibody that During was attempting to express in a plant. *Id.* (referring to Wood et al.) The few controls used by During in the tissue printing experiments were wholly insufficient under the circumstances to support During's assertion of success.

The During dissertation also includes immunogold electron microscopic analysis of his transgenic plant cells, apparently with the same antibodies used in the Western blotting and tissue printing experiments. The Examiner is referred to the Lerner declaration § 18 for a detailed explanation of During's immunogold results. Lerner takes issue with During's conclusion that the immunogold results indicate successful assembly of the B1-8 antibody in plants. First, Lerner notes that the heavy chain again was not detected and because areas of the cell that were immunogold labeled with the light chain reagent were not the same areas that were immunogold labeled with the Ac38 reagent (allegedly specific for assembled B1-8). Lerner declaration, ¶ 18. It stands to reason that for assembly to have occurred, the two chains should be co-localized to at least one area of the cell. Furthermore, During failed to observe immunogold labeling in regions of the cell that one would normally have expected antibody assembly to have occurred if assembly were in fact possible in plant cells. Lerner declaration, ¶ 19. Indeed, During observed immunoreactivity inexplicably in chloroplasts with the Ac38 antibody but not in the golgi apparatus or vesicles as others have observed previously for secreted proteins including antibodies. Unusual results might be acceptable if plant cells were capable of antibody assembly in unique and previously unknown ways, however, unusual results cannot make up for the lack of controls in other experiments.

Lerner concludes that a person skilled in the art of immunology or protein expression, circa 1988/1989, would not have reasonably believed the assertion of the During dissertation that plant cells could be used to process and assemble an antigen-specific immunoglobulin. Lerner declaration, ¶ 22. Lerner bases this opinion on During's failure to perform critical controls to support his conclusions and to explain his inconsistent results. Also, the Ac38 antibody which underlies virtually all of the support for During's assertion cannot be used, according to Lerner, to prove that NP antigen specific binding was present in plant cells. Lerner declaration, ¶ 22. Thus, even if During had done the proper antigen inhibition controls, much more would have been needed, according to Lerner, to overcome the prejudice in the art. *Id.*

It is also Lerner's opinion that even if there were no prejudice in the art, During's conclusions would still not have been accepted. This view is based in part on Lerner's

extensive experience as an editorial board member of more than ten scientific journals and an official reviewer for hundreds articles submitted for publication. Although During eventually published his antibody work in a peer-reviewed journal (i.e., Plant Molecular Biology), this occurred after the inventors of the above-captioned application published their work (1989 Nature article). Furthermore, as noted by Lerner, During's publication discusses the earlier publication by the inventors Hiatt and Hein at some length, describing it as a successful demonstration of antibody expression in plants. Lerner declaration, ¶ 22. In Lerner's opinion, had During not been able to support his work with the earlier publication by Hiatt and Hein, During's antibody expression experiments most likely would have been deemed unacceptable for publication. Lerner credits the inventors of the instant patent application, not During, as the first to convincingly demonstrate assembly of an antigen-specific immunoglobulin in plant cells.

The teachings of the During dissertation are equally if not more defective with respect to expressing an antigen-specific single polypeptide form of immunoglobulin in plants. The heavy chain variable region of the single polypeptide must assemble with the light chain variable region portion in order to achieve an antigen specific immunoglobulin. Lerner declaration, ¶ 24. Furthermore, as noted by Lerner, "I could find nothing in the During dissertation that addresses expression of a single polypeptide form of immunoglobulin, such as an sFv fragment." *Id.* It was the inventors of the above-captioned patent application, not During, who were the first to describe assembly of an antigen-specific sFv in plant cells.

These readily apparent deficiencies in the During dissertation with respect to assembly of a dual H/L or single immunoglobulin H/L polypeptide are not in any way cured by the teachings of Stolle. As already discussed, Stolle at most teaches avian immunization by conventional means. Thus, in view of all the above, the Examiner is respectfully requested to reconsider and withdraw the rejection of the claims for obviousness.

Applicant believes that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is urged to contact the undersigned by telephone to address any outstanding issues standing in the way of an allowance.

Respectfully submitted,

Date: March 13, 2002

By _____

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

13. (Twice amended) A method of passively immunizing a human or non-human animal subject against a preselected antigen [ligand], comprising administering to said subject a prophylactic amount of [a biologically functional] an antigen-specific immunoglobulin molecule which specifically binds to [~~and neutralizes~~] said preselected antigen [ligand] or immunologically active fragment thereof, wherein the immunoglobulin molecule is isolated from plant cells containing nucleotide sequence encoding [~~one or more biologically functional immunoglobulin product not normally produced by the plant~~] an immunoglobulin heavy chain polypeptide and an immunoglobulin light chain polypeptide wherein said nucleotide sequence also encodes a leader sequence for each polypeptide; and [biologically functional] antigen-specific immunoglobulin product encoded by said nucleotide sequences, wherein each [~~nucleotide sequence encoding an immunoglobulin polypeptide encodes a~~] leader sequence [~~forming~~] forms a secretion signal that is cleaved from each of said immunoglobulin heavy chain and light chain polypeptide following proteolytic processing[~~, and wherein said immunoglobulin is free from detectable sialic acid residues~~].

18. (Twice Amended) The method of claim 13, wherein said immunoglobulin heavy chain is a fragment of a full-length heavy chain [~~is an antibody or an immunologically active fragment thereof~~].

19. (Twice Amended) The method of claim 13, wherein said immunoglobulin light chain is a fragment of a full length light chain [~~is secretory IgA or an immunologically active fragment thereof~~].

20. (Amended) The method of claim 13, wherein said preselected antigen [ligand] is from a pathogen [antigen].

22. (Amended) The method of claim 20, wherein the pathogen is E. Coli, Salmonellae, Vibrio cholerae, or Salmonellae typhimurium[~~, or Streptococcus mutants~~].

23. The method of claim 13, wherein the plant [is] cells are from a monocot.

24. The method of claim 13, wherein the plant [is] cells are from a dicot.

29. The method of claim 13, wherein the immunoglobulin [~~molecule~~] heavy chain comprises at least a portion of a constant region and said constant region is from an IgA [~~molecule~~] heavy chain.

30. The method of claim 13, wherein the immunoglobulin [~~molecule~~] heavy chain comprises at least a portion of a constant region and said constant region is from an IgG [~~molecule~~] heavy chain.